

WHAT IS CLAIMED IS:

1                   1.       A method of screening an individual for increased risk of low  
2 folate status, said method comprising detecting a mutation in a human glutamate  
3 carboxypeptidase II (GCPII) gene in a biological sample from said individual, wherein  
4 detection of the mutation is indicative of decreased ability to hydrolyse a terminal  
5 glutamate residue of a folypoly- $\gamma$ -glutamate, which decreased ability is associated with  
6 low folate status.

1                   2.       The method of claim 1, wherein the mutation is a single nucleotide  
2 polymorphism.

1                   3.       The method of claim 3, wherein the single nucleotide  
2 polymorphism causes an amino acid substitution of H475Y.

1                   4.       A method of claim 1 wherein the mutation is detected by  
2                   (a) amplifying the GCPII gene, or a portion thereof containing the  
3 mutation, with a set of primers to provide an amplified product,  
4                   (b) sequencing the amplified product to obtain a sequence, and  
5                   (c) comparing the sequence of the amplified product with a known  
6 sequence of a wild-type GCPII gene,

7                   wherein a difference between the sequence of the amplified product and  
8 the sequence of the wild-type GCPII gene indicates the presence of a mutation.

1                   5.       A method of claim 4, wherein said amplification is by polymerase  
2 chain reaction.

1                   6.       A method of claim 4, wherein said sequencing is performed by  
2 detecting the incorporation of a nucleotide into a strand complementary to a template  
3 strand by detecting the presence of a pyrophosphate released from the incorporated  
4 nucleotide.

1                   7.       A method of claim 1 wherein the mutation is detected by  
2                   (a) amplifying exon 13 of the GCPII gene with a set of primers to  
3 provide an amplified product,  
4                   (b) sequencing the amplified product to obtain a sequence, and

5 (c) comparing the sequence of the amplified product with a known  
6 sequence of exon 13 of a wild-type GCPII gene,  
7 wherein a difference between the sequence of the amplified product and  
8 the sequence of the wild-type GCPII gene indicates the presence of a mutation.

1 8. A method of claim 7, wherein said primers are  
2 5'-CATTCTGGTAGGAATT TAGCA-3' and 5'-AAACACCACCTATGTTTAACA-3'.

1 9. A method of claim 7, wherein said amplification is by polymerase  
2 chain reaction.

1 10. A method of claim 7, wherein said sequencing is performed by  
2 detecting the incorporation of a nucleotide into a strand complementary to a template  
3 strand by detecting the presence of a pyrophosphate released from the incorporated  
4 nucleotide.

1 11. A method of claim 1, wherein said mutation is detected by  
2 hybridizing DNA from said individual to a test nucleic acid under stringent conditions.

1 12. A method of claim 11, wherein either said DNA from said  
2 individual or said test nucleic acid is immobilized on a solid support.

1 13. A method of claim 1, wherein said mutation is detected by  
2 (a) amplifying exon 13 said GCPII gene,  
3 (b) subjecting said amplified exon 13 to digestion by restriction  
4 enzymes,  
5 (c) separating the resulting restriction products to form a pattern of  
6 restriction fragment lengths, and  
7 (d) comparing the pattern of restriction fragment lengths to a  
8 pattern of restriction fragment lengths formed by subjecting amplified exon 13 of a wild-  
9 type GCPII gene to the same restriction enzymes.

1 14. A method of claim 13, wherein said separation of the restriction  
2 products is by gel electrophoresis.

1 15. A method of claim 13, wherein the restriction enzyme is AccI.

1                   16.     A method of claim 15, wherein the pattern of restriction fragments  
2 of exon 13 of the GCPII gene of the individual shows restriction fragments selected from  
3 the group consisting of: 141 bases and 103 bases.

1                   17.     A method of claim 1, wherein said mutation is detected by  
2 specifically binding an antibody to a truncated product of the GCPII gene, wherein the  
3 specific binding of the antibody to the truncated gene product is indicative of a mutation  
4 impairing the ability of the GCPII gene product to digest a dietary folate.

1                   18.     A method of claim 17, wherein detection of said specific binding of  
2 said antibody and said truncated gene product is by ELISA.

1                   19     A method of screening an individual for increased risk of low  
2 folate status comprising

3                   (a) performing reverse transcriptase-PCR on mRNA from intestinal cells  
4 of the individual to amplify products of a GCPII gene, and

5                   (b) determining the ratio of a variant product in which 93 bases of exon 18  
6 are deleted to a normal product of the GCPII gene,

7                   wherein a ratio of the variant form to the normal form greater than 1:3  
8 indicates the individual is at increased risk of low folate status.

1                   20.     A mutation in a GCPII gene which impairs the ability of a product  
2 of the gene to hydrolyse a conjugated folate to release folic acid compared to a product of  
3 a wild-type GCPII gene.

1                   21.     A mutation of claim 20, wherein the ability of a product of the gene  
2 to hydrolyse a conjugated folate is reduced by 20 percent or more compared to a product  
3 of a wild-type GCPII gene.

1                   22.     A mutation of claim 20, wherein the mutation is a 93-base deletion  
2 resulting from the elimination of exon 18.

1                   23.     The mutation of claim 20, wherein the mutation is a single  
2 nucleotide polymorphism.

1                   24.     The mutation of claim 23, wherein the single nucleotide  
2 polymorphism causes an amino acid substitution of: H475Y.

1                   25.     A kit for the detection of a woman at increased risk for bearing a  
2 child with a neural tube defect, comprising:

- 3                   (a) a container, and  
4                   (b) primers for amplifying a GCPII gene or portion thereof.

1                   26.     A kit of claim 25, further comprising instructions for detecting a  
2 mutation in the GCPII gene resulting in decreased ability of a product of the GCPII gene  
3 to hydrolyze a conjugated folate compared to the product of a wild-type GCPII gene.

1                   27.     A kit of claim 25, further comprising an AccI restriction enzyme.

1                   28.     A kit for the detection of an individual at increased risk for low  
2 folate status, comprising:

- 3                   (a) a container, and  
4                   (b) primers for amplifying a GCPII gene or portion thereof.

1                   29.     A kit of claim 28, further comprising instructions for detecting a  
2 mutation in the GCPII gene resulting in decreased ability of a product of the GCPII gene  
3 to hydrolyze a conjugated folate compared to a product of a wild-type GCPII, wherein  
4 detection of such a mutation indicates the individual is at increased risk for low folate  
5 status.

1                   30.     A kit of claim 28, further comprising an AccI restriction enzyme.